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ENDOTHELIAL DIFFERENTIATION: MOLECULAR MECHANISMS OF SPECIFICATION AND HETEROGENEITY

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Abstract

A complex and diverse vascular system is requisite for the survival of higher organisms. The process of vascular development is highly regulated involving the de novo formation of vessels (vasculogenesis), followed by expansion and remodeling of the primitive vasculature (angiogenesis), culminating in differentiation of endothelial phenotypes as found in the mature vascular system. Over the last decade significant advances have been accomplished in understanding the molecular regulation of endothelial cell development and differentiation. Endothelial development, in particular the mechanisms in play during vasculogenesis and angiogenesis, is discussed in a sister review to this article. This review highlights the key pathways governing in endothelial differentiation with a focus on the major molecular mechanisms of endothelial specification and heterogeneity.

Keywords

Vascular biology; endothelium; angiogenesis; developmental biology; endothelial differentiation

INTRODUCTION

In order to distribute oxygen, nutrients, and paracrine factors to far corners of a multicellular organism, a closed-loop circulatory system must be formed and connected to a pump very early in development. The afferent loop– the arterial system– must be able to endure high pressure, pulsatile blood flow and accomplish tissue-specific delivery of circulating materials. Uninterrupted return of blood to the pumping chamber must be maintained under the low pressure, low shear stress, high-capacitance conditions of the venous system. The diverse functions of a continuous system requires specialization of components of the system, and the heterogeneity of endothelial cells (ECs) lining the lumen of the system play a large part in creating this specialization.

Until the late 1990's the initial driving force in creation of heterogeneous EC phenotypes was thought to be the exposure of those cells to flowing blood. We now have data demonstrating that EC heterogeneity, while retaining the plasticity to alter under a changing environment, has a genetic component that comes into play perhaps even before hemangioblasts differentiate into ECs and hematopoietic cells. Elegant experiments in zebrafish and mouse embryos have provided the majority of the molecular data that drive

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our current understanding of EC heterogeneity. Human studies are largely limited to *in vitro* experiments with human-derived cells/tissues or to correlations with human vascular disease. Thus, the data reviewed below is generally from fish or rodent experiments. Unless otherwise noted, support of the molecular mechanisms described below was accomplished through classic genetic analyses with over-expression or knockdown of the molecule of interest and subsequent assessment of vascular consequences. Absolute concordance between the fish, rodent, and human systems has not been demonstrated. The aim of this review is to synthesize the available data in order to provide a general overview of the (vertebrate) pathway(s) to endothelial heterogeneity. For those wishing detailed experimental and system-specific discussions, several excellent recent reviews are available[1–6].

MOLECULAR MECHANISMS OF SPECIFICATION

Vascular progenitor cells (angioblasts) originate in the lateral plate mesoderm and migrate to a midline position just ventral to the notochord, forming the inner cell mass (ICM). The ICM gives rise to both blood and ECs. Cell-tracing experiments in zebrafish suggest that the arterial-venous cell fate decision of angioblasts is made even prior to migration[7]. The major embryonic vessels are formed by the coalescence of angioblasts to form the dorsal aorta (DA), which lies ventral to the notochord, and the posterior cardinal vein (PCV), located just below and parallel to the dorsal aorta[3, 8]. In addition to a genetic predestination of the angioblasts, the response to a VEGF gradient initiates a hierarchy of signaling events that culminates in arterial vs. venous EC differentiation (illustrated in Figure 1).

Arterial Specification

At the top of the hierarchy is the secreted ligand sonic hedgehog (Shh). Shh is a pleiotropic molecule, with diverse roles in embryogenesis and patterning. It is expressed in the notochord and results in expression of VEGF by somites bordering the developing vessels. The exact mechanism of mediation of the Shh signal in this instance is unknown; classically, it acts through its transmembrane receptor, patched-1, to increase VEGF activity and thus arterial specification[4, 9, 10]. However, there is evidence that Shh also increases VEGF by regulating expression of the calcitonin receptor-like receptor (crlr) expressed in somites and arterial progenitors[11, 12]. Diffusion of VEGF from the somites to the developing vessels creates a gradient with higher levels of VEGF near the DA and lower levels at the PCV. In vascular plexuses, increased VEGF expression leads to an increase in the arterial:venous ratio. VEGF acts through one of several VEGF receptor tyrosine kinases to activate, amongst other targets, the PLCγ1-MEK/ERK signaling pathway – solely in the dorsal angioblasts of zebrafish [2, 13, 14]. *In vitro* experiments using mouse-derived EC suggest that ERK can contribute to activation of the Foxc1 and Foxc2 transcription receptors that subsequently upregulate expression of members of the Notch pathway *in vivo* [15, 16]. Activation of the Notch pathway is a defining characteristic of arterial EC. The Notch family is composed of four receptors (Notch1 to Notch4) and 5 ligands (Jagged-1 and -2 and Delta-like ligands (Dll) 1 to 4). In mice, Notch1, Notch4, Jag1, Jag2, and Dll4 are all expressed in arterial but not venous ECs [3]. When Notch is activated, the intracellular domain is cleaved and translocates to the nucleus where it acts as a cofactor to upregulate transcription. Integrated expression of Notch genes during vessel development is required for appropriate vessel identity. Hemizygous deficiency of Dll4 is embryonic lethal in mice due to abnormal arterial development [17]. While Dll4 is essential for initiation of the arterial development program, Dll1 is required for maintenance [5, 18]. Alterations in levels of downstream targets of Notch lead to loss of arterial markers and arterial/venous fusion (compound Hey1/Hey2 mutant in mice;[19, 20]) and localized defects in the dorsal aorta (gridlock (grl) deficiency in zebrafish [21]). The Sox7 and Sox18 gene products may regulate arterial-venous specification

by acting upstream of grl[22–24]. In fact, arteriovenous malformations develop when Notch signaling is either reduced or constitutively active.

The ephrinB2 ligand and its cognate receptor, EphB4, are differentially expressed in the arterial and venous ECs, respectively, of the mouse primary vascular plexus prior to the onset of circulation [25, 26]. This seminal discovery, in 1998, provided the first evidence that arterial-venous identity is genetically predetermined. Although this ligand-receptor pair have distinct cellular locations, interactions between the two are required for proper vascular development/remodeling. The ephrin-Eph subclass of receptor tyrosine kinases can participate in an unusual bidirectional signaling process. Forward signaling (ephrin ligand to Eph receptor) is initiated by ephrin ligand engagement and activates the receptor kinase domain. Reverse signaling (Eph receptor to ephrin subclass B ligand) leads to phosphorylation of tyrosine residues in the cytoplasmic domain of the ligand. This large subfamily of signaling molecules regulates a variety of morphogenetic processes in different tissues (reviewed in [1]). The lack of ephrin B2 or EphB4 does not impair the initial specification of arteries and veins, but ephrinB2-EphB4 signaling is required for maintenance of the arterial-venous interaction. Mouse mutants defective for the pair lose the differentiation of blood vessels into morphologically distinguishable arteries and veins [25–27]. This limited role lead to the subsequent identification of upstream factors, such as Notch, in the process of arterial/venous EC differentiation.

Expression of the neuropilins (nrp) is temporally related to the expression of the ephrins and also displays a restricted pattern of expression. Nrp-1 is found exclusively in cells fated to be arterial, and Nrp-2 in venous [28]. In mice with a CD1 background, Nrp-1 deficiency leads to abnormal vascular network formation and embryonic lethality at E13.5. In the C57Bl/6 background, the vascular defects are not apparent until after the onset of blood flow. In these mice, deficiency abrogates the normal vascular remodeling that occurs coincident with the initiation of flowing blood (E10.5) [29–31]. The neuropilins were previously identified as cell surface receptors for semaphorins. In this system, Nrp-1 functions at least in part by acting as a co-receptor for VEGF and facilitating VEGF signaling in concert with VEGF receptor-2 [32].

Venous Specification

Unlike arterial specification, details of the molecular mechanisms controlling venous specification are still largely unknown. Exposure to lower VEGF concentrations is likely to be important as a negative regulator of arterial specification. *In vitro* experiments have shown that higher concentrations of VEGF (50ng/ml) induces expression of arterial markers such as ephrinB2 and Nrp-1 in embryonic stem cells whereas low VEGF concentrations (2ng/ml) led to expression of venous endothelial markers [33]. In zebrafish, loss of Shh signaling leads to loss of the arterial marker Ephrin-B2a and greater amounts of the venous marker Flt4[34]. Thus, the greater distance of the PCV from the VEGF-spewing somites (as compared to the DA) may contribute to its differentiation into a venous structure (reviewed in [3]). *In vitro* studies have shown that VEGF activates a plethora of signaling pathways including PI3K/Akt pathway, which can act in concert with VEGF-activated ERK; however, in regard to arterial EC specification, *in vitro* and *in vivo* experiments suggest that the MEK/ERK and PI3K/AKT pathways may have competing roles [35]. Specifically, activation of AKT can inhibit the activity of MEK/ERK in zebrafish, thus steering EC away from arterial specification [14]. (Replication of Notch inhibition by PI3K in *in vitro* experiments has not been accomplished; loss of the complexities of the *in vivo* context is considered a potentially important factor [36, 37].) AKT is also hypothesized to induce chicken ovalbumin upstream promoter-transcription factor (COUP-TFII) expression [2]. COUP-TFII is specifically expressed in venous ECs and is a genetic determinant of venous specification. While compound homozygous mutants for Foxc1 and Foxc2 lack arterial specific genes, they do

express the venous marker COUP-TFII[16]. As a nuclear orphan receptor, the natural ligand for COUP-TFII is retinoic acid. Activation of COUP-TFII by retinoic acid suppresses Notch signaling, potentially at the level of NRP-1 expression, thus releasing factors such as EphB4 from Notch-mediated repression, and conferring a venous EC phenotype [38]. COUP-TFII directly regulates expression of Nrp-2, leading both to its restricted expression in venous (vs. arterial) ECs and preparing cells for differentiation into lymphatic EC [39].

Lymphatic Specification

Lymphatic EC (LEC) are derived from lymphatic vessel hyaluronan-1 receptor-1 (LYVE-1) positive subpopulations of cells in the cardinal vein which bud off to form primary lymphatic sacs. LYVE-1 is a specific marker for cells capable of becoming LEC, but is not required for normal lymphatic development[40, 41]. By molecular mechanisms not yet elucidated, the transcription factor SRY box 18 (Sox18) is induced in murine LYVE-1 positive venous cells that will commit to the LEC lineage. Sox18 directly induces expression of Prox1, the master regulator of LEC identity [42]. Recent data suggests that Prox1 is required for both initiation and maintenance of the LEC phenotype. Prox1 deficient EC can bud from the cardinal vein, but they display abnormal VEGF-induced migration and do not express LEC markers [43, 44]. Recent data shows that lymphatic sprouting, specifically (versus angiogenic), is regulated in zebrafish by the PDZ domain-containing scaffold protein synectin; *in vitro* experiments with human dermal LECs suggest that synectin functions, in part, by regulating Sox18-mediated induction of Prox1[45]. Pedrioli and colleagues used microarray analysis to identify human blood EC- or LEC-specific microRNAs, and found that miR31 suppresses lymphatic differentiation[46]. Mechanistic analysis demonstrated that the inhibitory effect is partially mediated via direct repression of Prox1.

Prox1 mediates upregulation of important regulators/markers of lymphatic differentiation, including the VEGF receptor, VEGFR-3. VEGFR-3 is expressed in all EC during early development; however, its expression is enhanced in cells committed to the LEC lineage, and as the lymphatic system develops, becomes largely restricted to LECs[47]. Activation of VEGFR-3, in particular, by VEGF-C is required for lymphatic development [48]. It is proposed that the VEGFR-3 co-receptor Nrp-2 increases the response of LEC to VEGF-C by a mechanism analogous to the interaction of Nrp-1 and VEGFR-2 in arterial EC (as reviewed in[6]).

The presence of COUP-TFII in EC may well be a determinant factor for the venous origin of cells destined to become LEC. Sox18 cooperates with COUP-TFII to promote expression of Prox-1[42]. Prox1 appears to act in concert with COUP-TFII in enhancing expression of VEGFR-3, and by upregulating the VEGFR-3 co-receptor Nrp-2, COUP-TFII enhances VEGFR-3 activity [39].

Plasticity of Vascular Specification

While the initial molecular identity of ECs is genetically predetermined, there is significant plasticity in subsequent arteriovenous differentiation. Physiological requirements and hemodynamic influences can reverse the phenotype of an apparently committed cell. ECs in transplanted arterial and venous vessel grafts can change identity, completely switching their expression profile to match the host vessel [27, 49]. Forced over-expression or loss-of-function of critical molecular determinants of specification can also reprogram a differentiated EC. For example, Notch over-expression in the venous compartment results in arterialization with upregulation of arterial EC markers while inhibition of Notch signaling in the arterial compartment results in loss of arterial fate and upregulation of venous markers[50]. Ablation of EC COUP-TFII allows veins to acquire arterial characteristics and express arterial markers[38]. The mature LEC phenotype is highly plastic[51, 52]. As discussed above, constant

expression of Prox1 maintains the mature differentiated LEC state. Conditional embryonic, post-natal, or adult deletion of Prox1 in mice results in the appearance of blood-filled lymphatic vessels and down regulation of LEC markers with the concomitant ectopic expression of blood EC markers[53]. Conversely, ectopic over-expression of Prox1 in cultured blood ECs leads to the acquisition of LEC identity[54, 55]. An exquisite feedback regulatory equilibrium exists among the three major EC fate regulators (Notch, COUP-TFII, and Prox1) that directs the plasticity in arteriovenous-lymphatic cell fate[56].

MOLECULAR MECHANISMS OF HETEROGENEITY

Beyond the specification to arterial, venous, or lymphatic fate, it is currently recognized that ECs undergo further differentiation specific to the vascular bed or organ in which they reside. This phenotypic heterogeneity is the primary mechanism by which the endothelium carries out myriad vital functions including: control of microvascular permeability, vessel wall tone, coagulation and anticoagulation, inflammation, and angiogenesis[1, 57, 58]. Endothelial heterogeneity is also responsible for the varied and diverse responses across differing vascular beds to pathologic stimuli and disease states[59–61].

Structural Classifications

Based on structural content, the endothelium has been classically characterized into three main structural types: continuous, which is further subdivided into fenestrated or non-fenestrated, and discontinuous (or sinusoidal)[57, 62] (illustrated in Figure 2A).

Continuous Non-fenestrated—Continuous non-fenestrated endothelium is found predominantly in arteries, veins, and capillaries of the brain, skin, muscle, heart, and lung. Tight junctions and adherens junctions are the two main types of barrier forming intracellular junctions found in this type of endothelium[63, 64]. Their expression is variable across the endothelial tree with higher expression in the continuous endothelium of arterioles when compared to capillaries and venules. Molecules cross this endothelium by the active process of transcytosis, which is mediated by specialized structures including caveolae and vesiculo-vacuolar organelles (VVOs). Caveolae, flask-shaped membrane bound vesicles (~70nm in diameter) that usually open to the luminal or abluminal side[65, 66], are present in all types of endothelium but are highest in capillaries that contain continuous non-fenestrated endothelium[67]. Caveolin-1 is the main structural component of caveolae and is regulated by distinct transcriptional mechanisms in ECs[68]. VVOs also contain caveolin-1 and are focal collections of membrane bound vesicles of variable size that span the cytoplasm of the ECs[69] and are mostly found in venules with continuous non-fenestrated endothelium[70].

Continuous Fenestrated—Fenestrae are transcellular pores (~70nm in diameter) that extend through the full thickness of the EC and are thought to allow rapid exchange of molecules between the circulation and the surrounding tissue[71]. The majority of fenestrae contain a thin diaphragm across their opening which acts as a molecular filter. The type II membrane glycoprotein plasmalemmal vesicle associated protein-1 (PV-1) is currently the only molecular protein localized to the diaphragm[72] and it has been discovered to be both necessary and sufficient for diaphragm formation in cultured ECs[73, 74]. Other diaphragm containing components of continuous endothelium include caveolae and transendothelial channels (TECs), which are patent pores spanning the EC from the luminal to abluminal side [75]. Compared to non-fenestrated endothelium, continuous fenestrated endothelium is more permeable to water and small solutes. This endothelium typically occurs in locations that are characterized by increased filtration or increased transendothelial transport and is

found in capillaries of all exocrine and endocrine glands, digestive tract mucosa, and kidney (e.g. glomeruli and a subpopulation of renal tubules).

Discontinuous Fenestrated—Discontinuous fenestrated endothelium is characterized by large heterogeneous fenestrae (100–200nm in diameter) without diaphragms[76]. It has few caveolae and contains clathrin-coated pits and vesicles, which play an important role in receptor-mediated endocytosis. This endothelium is found in certain sinusoidal vascular beds, most notably the liver and bone marrow which lack a well formed basement membrane.

The phenotypic heterogeneity of the endothelium of the various organ and vascular beds has been highly studied[1, 57, 58]. Remarkably, despite these detailed observations, the molecular mechanisms of endothelial heterogeneity remain largely unknown. In recent years, the study of ECs in several vascular beds have made significant strides in elucidating some of these molecular mechanisms.

Kidney Endothelium

The kidney is a highly vascular organ that contains a large degree of endothelial heterogeneity. Blood from the renal arteries reaches the kidney and branches into the afferent arterioles that enter into the glomerular tufts. The glomerular endothelium in conjunction with the basement membrane and underlying podocytes helps to form the glomerular filtration barrier which serves as both a size and charge selective filter[77, 78]. The glomerular endothelium actively synthesizes a 60–300nm thick gelatinous surface coat called glycocalyx (composed of proteoglycans, glycosaminoglycans, glycoproteins, glycolipids, and associated plasma proteins) that covers the luminal side and facilitates charge selectivity[77, 79]. The glomerular capillaries consist of a continuous fenestrated endothelium with large fenestrae that cover 20–50% of the entire endothelial surface [80]. A unique feature of the glomerular endothelium is that most fenestrae do not contain diaphragms. The majority of evidence suggests that the glomerular ECs lose their expression of PV-1 and fenestral diaphragms during the development and maturation of the glomerulus[81–83]. Importantly, recent studies of the glomerular endothelium have confirmed the initial observations that VEGF plays a role in the formation of EC fenestrations[84–86]. Cross talk between podocytes, expressing VEGF-A, and the glomerular endothelium, expressing VEGF receptors, has been shown to be important for the development and maintenance of fenestrae and barrier function[87–89]. Studies are ongoing investigating the detailed molecular pathways, but initial findings implicate activation of the small GTPases (i.e. Rho/Rac) and rearrangement of the actin cytoskeleton[83].

Efferent arterioles exit the renal glomeruli and terminate in the vasa recta. Endothelial heterogeneity of the vasa recta is critical for the countercurrent exchange that occurs in the medulla of the kidney[90]. The descending vasa recta (i.e. an arteriole entering the medulla) is lined by continuous non-fenestrated endothelium and contains large concentrations of urea transporters and aquaporin-1 water channels[91]. In contrast the ascending vasa recta (i.e. a vein exiting the medulla) is lined with fenestrated endothelium. This hetereogeneity allows for shuttling of osmotically active solutes between descending and ascending capillaries thereby maintaining the hypertonicity of the medulla that is essential for gradient mediated filtration in the kidneys. In addition this process helps to deliver nutrients and oxygen to the medullary tissue [91].

Brain Endothelium

The brain endothelium has an extremely specialized characteristic that allows it to selectively control permeability between blood and the central nervous system, thereby

forming the blood brain barrier (BBB)[92, 93]. This barrier function is primarily mediated by both a physical barrier due to tight interendothelial junctions and a highly selective transporter system. The hallmark feature of the endothelium of the BBB is a continuous non-fenestrated endothelium with few caveolae and high expression of tight junction proteins, namely occludin and members of the claudin family (claudin 1, 3, 5, and 12). As tight junctions form, the brain ECs also begin to express selective membrane transporter proteins such as glucose transporter type 1 (Glut-1) and members of the ATP binding cassette (ABC) transporter family (ABCB1/P-glycoprotein/MDR1 and BCRP/ABCG2). This differentiation and maturation process of acquiring the unique properties of the BBB is frequently called barriergenesis[94, 95].

The brain endothelium is surrounded by several other cell types including pericytes, astrocytes, and neurons, forming the neurovascular unit (NVU)[96], and interaction between multiple components of the NVU have been found to be necessary for proper formation and function of the BBB. Recent studies demonstrate that pericyte-EC interactions are necessary for BBB development by regulating the formation of tight junctions as well as controlling pinocytic transport vesicles in ECs[97, 98]. Additionally, the astrocyte-derived factor Src-suppressed C kinase substrate (SSeCKS) strengthens the BBB by decreasing VEGF expression and inducing Angiopoietin-1 (Ang-1), resulting in increased tight junction expression in brain ECs [99] (illustrated in Figure 2B).

Recently the canonical Wnt pathway has been discovered to play a major role in barriergenesis[100]. In these studies, the expression of Glut1 was induced by Wnt7a and Wnt7b[101, 102]. Additionally, Wnt signalling in ECs was necessary and sufficient for the induction and maintenance of BBB characteristics by increasing claudin 3 expression and inhibiting PV-1[103] (illustrated in Figure 2B). These recent findings in barriergenesis predict that the Wnt signaling pathway, which has been shown to play important roles in vascular morphogenesis, is likely to be involved in the mechanism of endothelial heterogeneity in other vascular beds and organs[104].

Aortic Endothelium

Endothelial heterogeneity exists not only between various organs and vascular beds but also within a single vascular bed. One of the most striking examples of this type of heterogeneity is the aortic endothelium, where variable blood flow dynamics results in the nonuniform distribution of atherosclerosis[105, 106]. Laminar blood flow in straight segments of the aorta induces factors such as endothelial nitric oxide synthase (eNOS) and thrombomodulin (TM), thereby conferring potent anti-thrombotic, anti-adhesive, and anti-inflammatory properties to the endothelium[107, 108]. Conversely, non-laminar or turbulent blood flow at areas where arteries branch or turn sharply reduces eNOS expression and induces adhesion molecules such as vascular cell adhesion molecule [VCAM]-1, resulting in an inflammatory endothelial phenotype[106, 109, 110]. Two members of the Kruppel-like factor transcription family (KLF2 and KLF4) are strongly induced by laminar flow, via the MEK/ERK5/MEF2 pathway, and have been shown to be key molecular mediators of flow-mediated endothelial heterogeneity[111] (illustrated in Figure 2C).

SUMMARY AND FUTURE DIRECTIONS

As we continue to unravel the molecular and physiological mechanisms that create the diversity of ECs, we hope to become increasingly able to harness the plasticity of the EC phenotype in order to modulate the pathophysiological events specific to various vascular beds. Such molecular insights may allow one to manipulate EC phenotype in the treatment of vasculo-centric disease states that are major sources of morbidity and mortality including atherothrombosis, sepsis, or tumorigenesis. Finally, advances in this area of biology may be

facilitated through establishment of improved models of vascular development and plasticity. While much of the work to date has relied on studies in zebrafish and mice, a recent study found that the mechanism of cell commitment in early embryos differs significantly between mice and cows[112]. In this regard, use of human iPS technology may allow for novel insights into human endothelial differentiation, specification, and heterogeneity.

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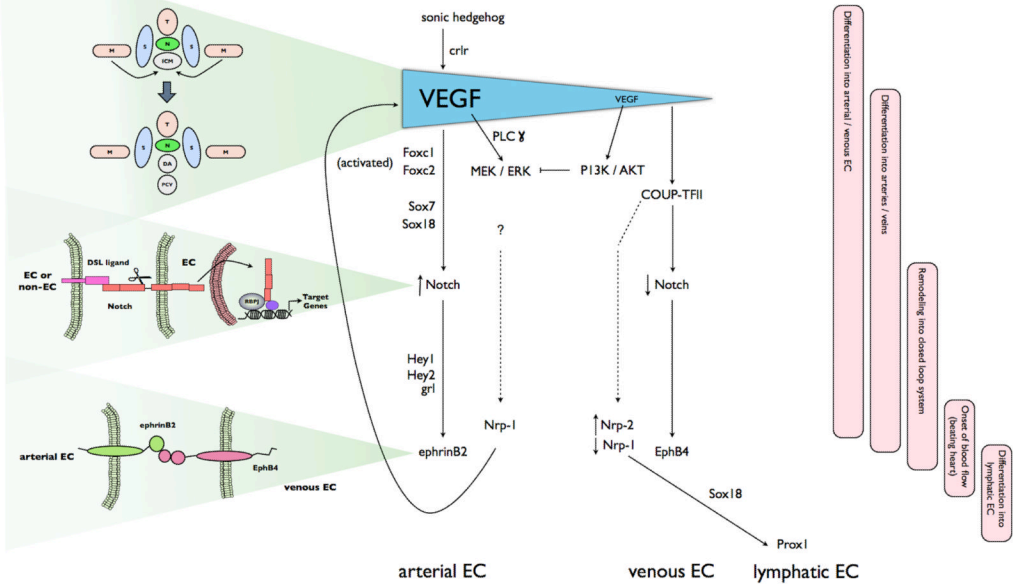

Figure 1.

A model for the proposed molecular pathways in arterial, venous and lymphatic specification in a developing embryo. Schematics on the left portion of the figure illustrate anatomic correlates of the molecular pathways described in the central flow diagram. The bars on the right portion of the figure highlight developmental correlates to the molecular pathways. Shh expression in the notchochord triggers VEGF expression by the somites and creation of a VEGF gradient. High levels of VEGF activate PLCγ and induce arterial specification by activating the MEK/ERK pathway through the VEGFR1-NRP1 receptor complex, and the PI3K/AKT pathway in cells residing further ventral. Arterial specification proceeds with activation of Foxc1/2 and Notch signaling, inducing ephrinB2 and Nrp-1expression. Nrp-1 creates a positive feedback loop by acting as co-receptor for VEGF. Venous specification occurs when ERK is suppressed, and COUP-TFII induced, by AKT. COUP-TFII inhibits arterial specification by down-regulating Notch and Nrp-1, allowing expression of venous-specific markers including Nrp-2. A subpopulation of venous EC subsequently express Sox18 and Prox1, resulting in lymphatic EC specification. The upper schematic highlights the migration of angioblasts and subsequent development, directed by VEGF concentrations, of the DA and PCV; the middle schematic the importance of activation of the Notch pathway by cleavage and nuclear translocation of the cytoplasmic domain of the receptor; and the lower schematic the requirement of cell-to-cell communication via ephrin-Eph receptor kinases in maintaining arterial/venous discrimination. T, neural tube; M, lateral mesoderm; S, somite; N, notochord; ICM, inner cell mass. Full details are found in the text.

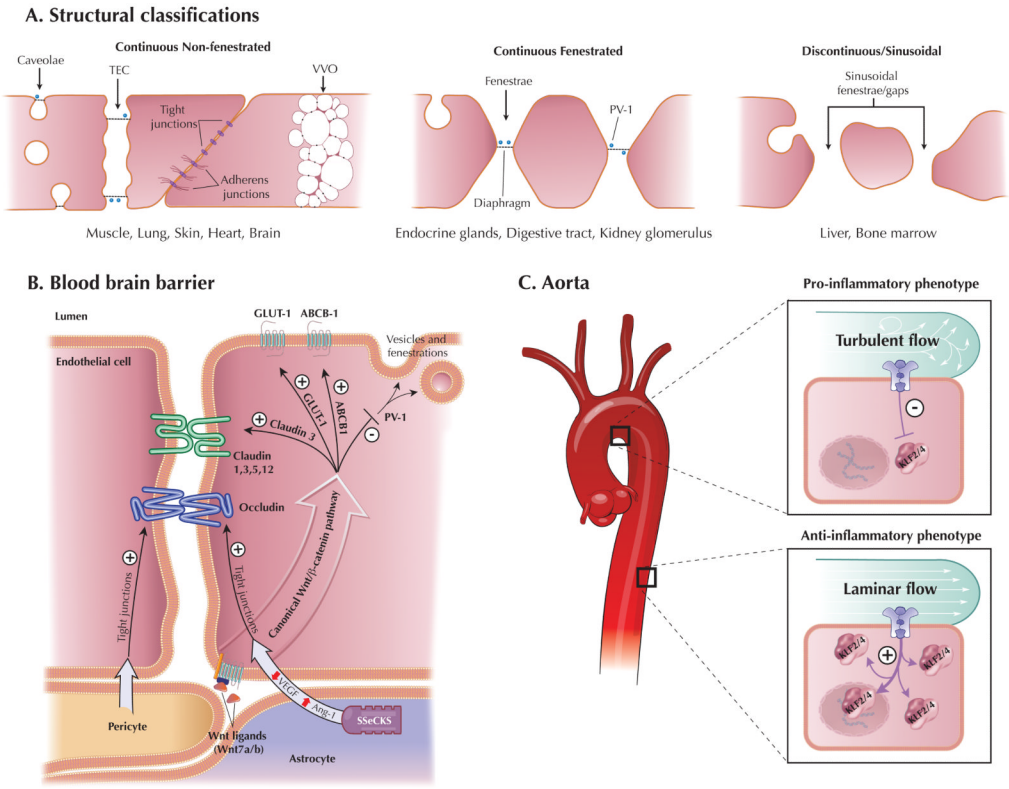

Figure 2.

Endothelial Cell Heterogeneity. A, Schematic diagram of the three main endothelial structural classifications (modified from [57]). TEC= transendothelial channel, VVO=vesiculo-vacuolar organelles. B, Schematic diagram of the molecular mechanisms of blood brain barriergenesis (modified from [100]). C, Heterogeneity of the aortic endothelium. Turbulent flow occurring in the lesser curvature of the aortic arch or at branch points in the vascular tree inhibits KLF2/4 expression resulting in a more pro-inflammatory endothelial phenotype. Whereas laminar flow occurring in linear segments upregulates endothelial KLF2/4 expression resulting in a more anti-inflammatory phenotype.